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Determination of Potential Genotoxic Impurities in Sorafenib Tosylate by UPLC Method

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ABSTRACTS: An isocratic reversed-phase UPLC method with UV detector has been developed for the determination of Methyl Tosylate, Ethyl Tosylate and Isopropyl Tosylate in Sorafenib Tosylate. These are potential genotoxic impurities and hence need to be controlled in Sorafenib Tosylate. The analysis was performed using RRHD Eclipsed Plus C18 UPLC column (50 x 2.1mm, 1.8µm) as a stationary phase with column oven temperature 40°C, and UV detection at 226nm. The separation was achieved using mobile phase comprising of 50mM Sodium Perchlorate in water and pH adjusted to 3.0 with glacial acetic acid and Acetonitrile in the volume ratio of 60.0:40.0. The method was optimized based on the peak shapes and resolution between Methyl Tosylate, ethyl Tosylate, propyl Tosylate and Sorafenib Tosylate. The method was validated as per International Conference of Harmonization (ICH) guidelines in terms of limit of detection (LOD), Limit of quantitation (LOQ), linearity, precision, accuracy, specificity, robustness and solution stability. The LOD and LOQ values were found to be 0.045µg/ml and 0.09µg/ml, respectively. The sample concentration were injected was 15mg/ml. The method is linear within the range of 0.09-0.3µg/ml for both the Impurities.

Key Words: Sorafenib Tosylate; Methyl Tosylate; Ethyl Tosylate; Propyl Tosylate; Cancer drugs; Genotoxic Impurity.

I. INTRODUCTION

Genotoxic compounds induce genetic mutations and/or chromosomal rearrangements and can therefore act as carcinogenic compounds [1]. These compounds cause damage to DNA by different mechanisms such as alkylation or other interactions that can lead to mutation of the genetic codes. In general, chemists employ the terms "genotoxic" and "mutagenic" synonymously; however, there is a subtle distinction. Genotoxicity pertains to all types of DNA damage (including mutagenicity), whereas mutagenicity pertains specifically to mutation induction at the gene and chromosome levels. Thus, the term “genotoxic” is applied to agents that interact with DNA and/or its associated cellular components (e.g. the spindle apparatus) or enzymes (e.g. topoisomerases) [2, 3]. Irrespective of the mechanism by which cancer is induced, it is now well agreed that it involves a change in the integrity or expression of genomic DNA. The majority of chemical carcinogens are capable of causing DNA damage, i.e., are "genotoxic" [4]. Moreover, a genotoxic compound also carries with it the carcinogenic effect which causes additional concern from the safety viewpoint.

Sulfonate salts are the most frequently used compounds in pharmaceutical developments. Salt formation is a useful technique for optimizing the physicochemical processing (formulation), biopharmaceutical or therapeutic properties of active pharmaceutical ingredients (APIs), and sulfonate salts are widely used for this purpose [5]. However sulfonic acids can react with low molecular weight alcohols such as methanol, ethanol, or isopropanol to form the corresponding sulfonate esters. In general, sulfonic acid esters are considered as potential alkylating agents that may exert genotoxic effects in bacterial and mammalian cell systems and possibly carcinogenic effects in vivo; thus, these compounds have raised safety concerns in recent times [6,7]. Sorafenib, an antineoplastic agent acts as protein kinase. Sorafenib inhibits tumor cell proliferation and the tumor cell vacularisation through activating the receptor tyrosine kinase signalling RAS/RAF/MEK/ERK cascade pathway. In this work we demonstrate the practical example for the analytical control of three genotoxic impurites in Sorafenib Tosylate. These impurities Methyl Tosylate, Ethyl Tosylate, Isopropyl Tosylate and Sorafenib Tosylate

were characterized and structure was deduced (Fig. 1-12). These impurities were observed to be process impurities. From the literature it was found out that these impurites are genotoxic [8-16]. The method is based on Ultra performance liquid chromatography (UPLC) for determination of Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate. The method was validated as per International conference of Harmonization (ICH) guidelines in terms of limit of specificity, linearity accuracy, detection (LOD), Limit of quantitation (LOQ), precision and solution stability [17].

II. MATERIAL AND METHODS

A. Drug and reagents

Pure Sorafenib Tosylate was purchased from G.M fine chemicals (Hydrabad, India). Standards of Methyl Tosylate, Ethyl Tosylate and Iso-propyl Tosylate were obtained from Sigma Aldrich. Analytical reagent (AR) grade Sodium perchlorate was purchased from Fluka (Banglore, India), acetic acid from Merck (Mumbai, India) and Acetonitrile from sigma Aldrich (Mumbai, India). Water for UPLC studies was obtained from milipore water purifying system.

B. Apparatus and equipment

LC was carried out on WATERS Aquity UPLC (USA) with photodiode array detector. The output signal was monitored and processed using Empower 2 software. In all the studies, separations were achieved on RRHD Eclipsed Plus C-18 column (50 mm x 2.6 mm i.d., particle size 1.8µm) procured from LCGC (Banglore, INDIA).

A pH/Ion analyzer (Labindia, made in) was used to check and adjust the pH of buffer solutions. Other small equipment were PCI sonicator (22L500/CC/DTC made in), precision analytical balance (MX5, Mettler Toledo, Schwerzenbach, Switzerland).

All the instruments used were calibrated and were used within the specified dyanamic range.

C. Preparation of mobile phase

The mobile phase comprised of 50mM Sodium Perchlorate in water and pH adjusted to 3.0 with glacial acetic acid and Acetonitrile in the volume ratio of 60:40.

D. Chromatographic conditions

The numbers of column chemistries like C8, Penta Fluro Phenyl, Phenyl were used during method development. The best separation was achieved on RRHD Eclipsed Plus C-18 column (50 mm x 2.1 mm i.d., particle size 1.8µm) using isocratic mixture of 50mM Sodium Perchlorate in water and pH adjusted to 3.0 with glacial acetic acid and Acetonitrile in the volume ratio of 60:40 with the flow rate set at 0.5ml/min and column temp. maintained at 40^{o}C. The injection volume was set 10µl and detector was set at a wavelength of 226nm.

E. Preparation of sample during method development

The diluent selected for dissolving Sorafenib Tosylate and the impurities was mobile phase. Stock solution of Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate impurities were prepared in diluent having concentration of 0.2mg/ml. Further 5 mL of each impurities stock solution were transferred to a single 100ml volumetric flask and diluted up the volume with diluent. Further transferred 2 ml this solution was transferred to 100ml volumetric flask and diluted up the volume with diluent. Sorafenib Tosylate sample solution were prepared in the concentration 15mg/ml. The concentration of Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate impurities was 13 ppm w.r.s Sorafenib.

F. Preparation of sample and impurities for validation

Stock solution of Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate impurities and Sorafenib were prepared in diluent. These stock solutions of impurities were further diluted with diluent to get the required concentration for validation studies.

III. RESULTS AND DISCUSSION

A. Method development and column selection

Characterization data and chemical structure of Sorafenib Tosylate and Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate impurities are shown in (Fig. 1-12). The production batch sample of Sorafenib Tosylate which was selected for validation studies.

Different mobile phase and stationary phases were employed to developed a suitable LC method for the quantitative determination of Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate impurities in Sorafenib Tosylate. A number of column chemistries supplied by different manufacturers and different mobile phase composition were tried to get good peak shapes and selectivity for the impurities present in Sorafenib. Poor peak shape and resolution was observed when Waters Acquity BEH C18 (50 x 2.1mm, 1.7µm) and mobile phase consisting of mixture of 0.1% triethylamine in water: Acetonitrile and Methanol (80:10:10 v/v/v) Sorafenib eluted at 5 min good separation, however peak shape of Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate impurities was not good. By using another attempt with mixture of mobile phase 0.1% Triethylamine, Acetonitrile and methanol (75:15:10 v/v/v/) and column Waters Acquity BEH C8 (50 x 2.1mm, 1.7µm), ethyl Tosylate eluted in close proximity to iso-propyl Tosylate.

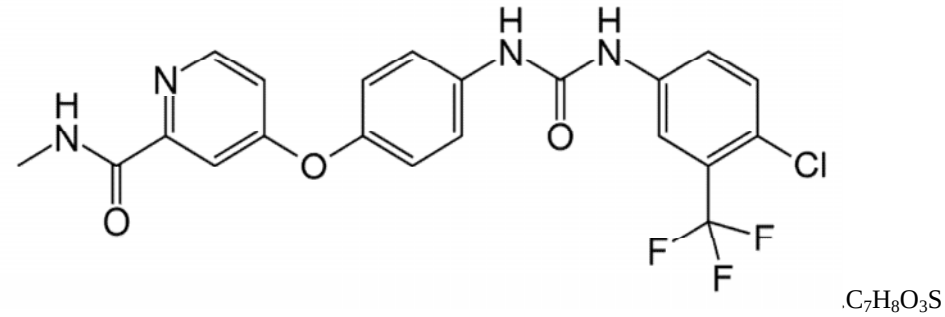

Fig. 1. Sorafenib Tosylate.

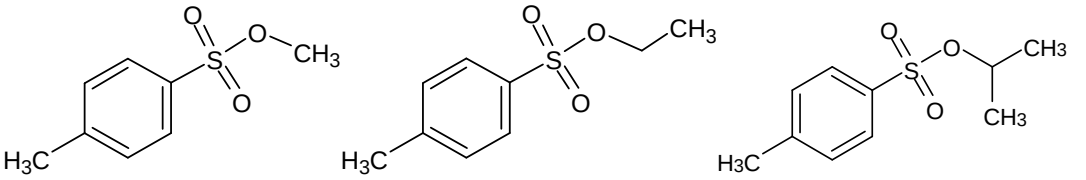

Fig. 2. Impurities.

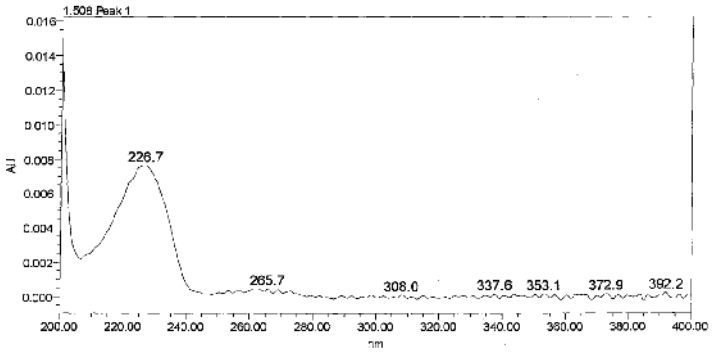

Fig. 3. Methyl Tosylate UV spectra.

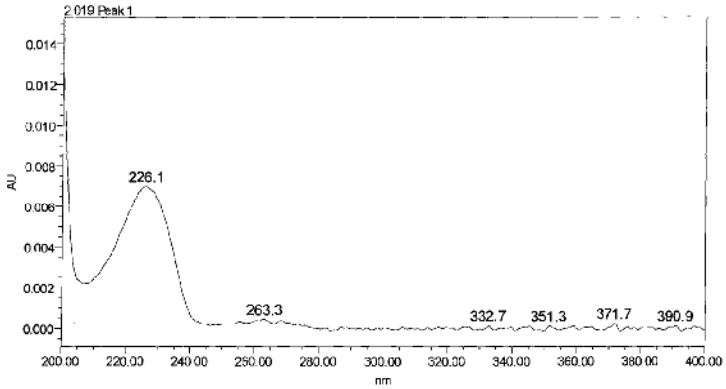

Fig. 4. Ethyl Tosylate UV spectra.

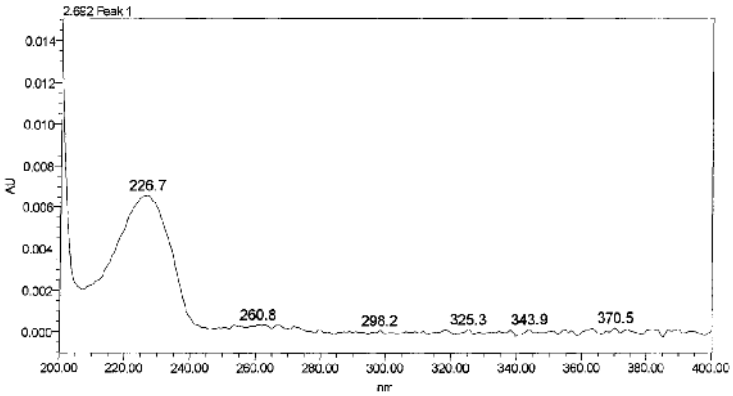

Fig. 5. Isopropyl Tosylate UV spectra.

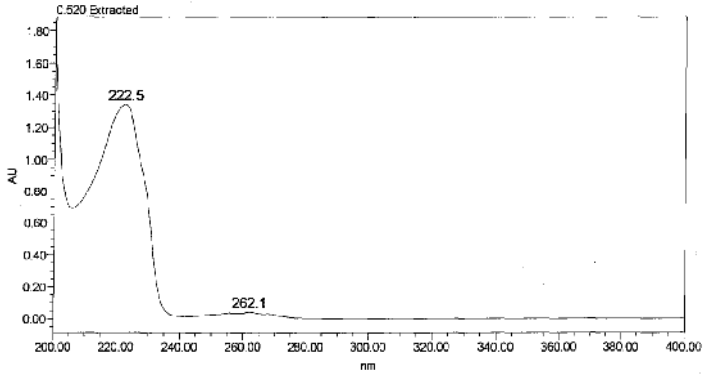

Fig. 6. Sorafenib Tosylate UV spectra.

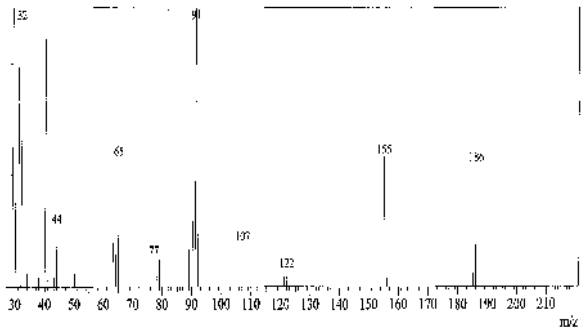

Fig. 7. Mass data for Methyl Tosylate.

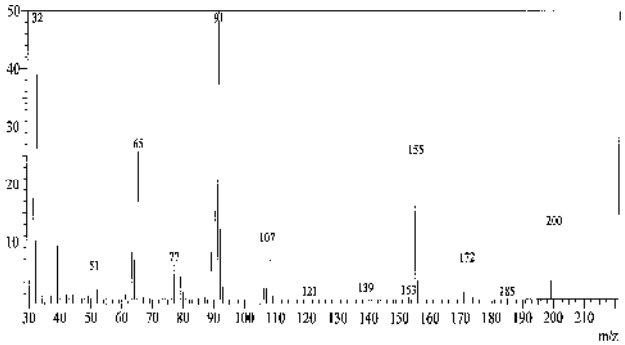

Fig. 8. Mass data for Ethyl Tosylate.

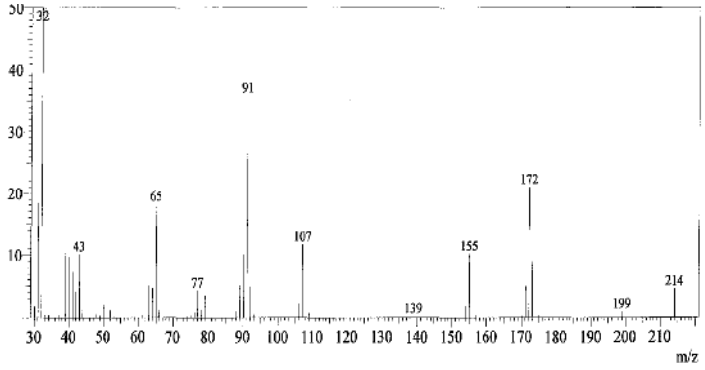

Fig. 9. Mass data for Isopropyl Tosylate.

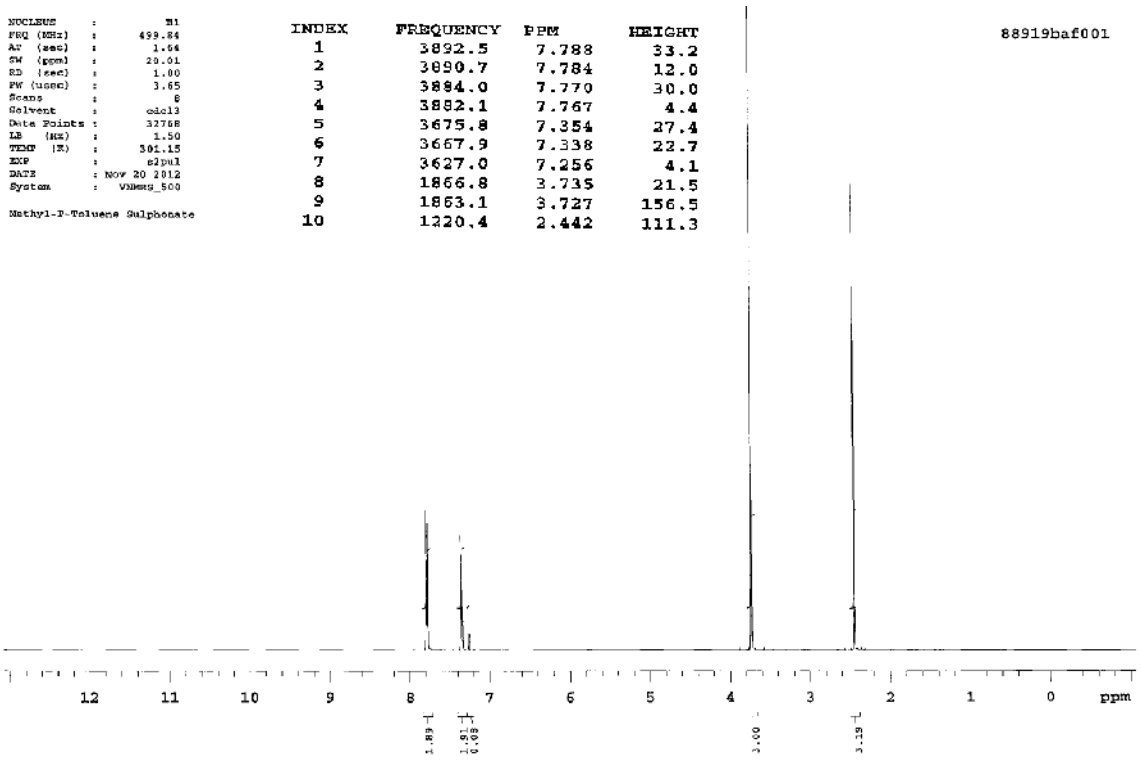

Fig. 10. Proton NMR for Methyl Tosylate.

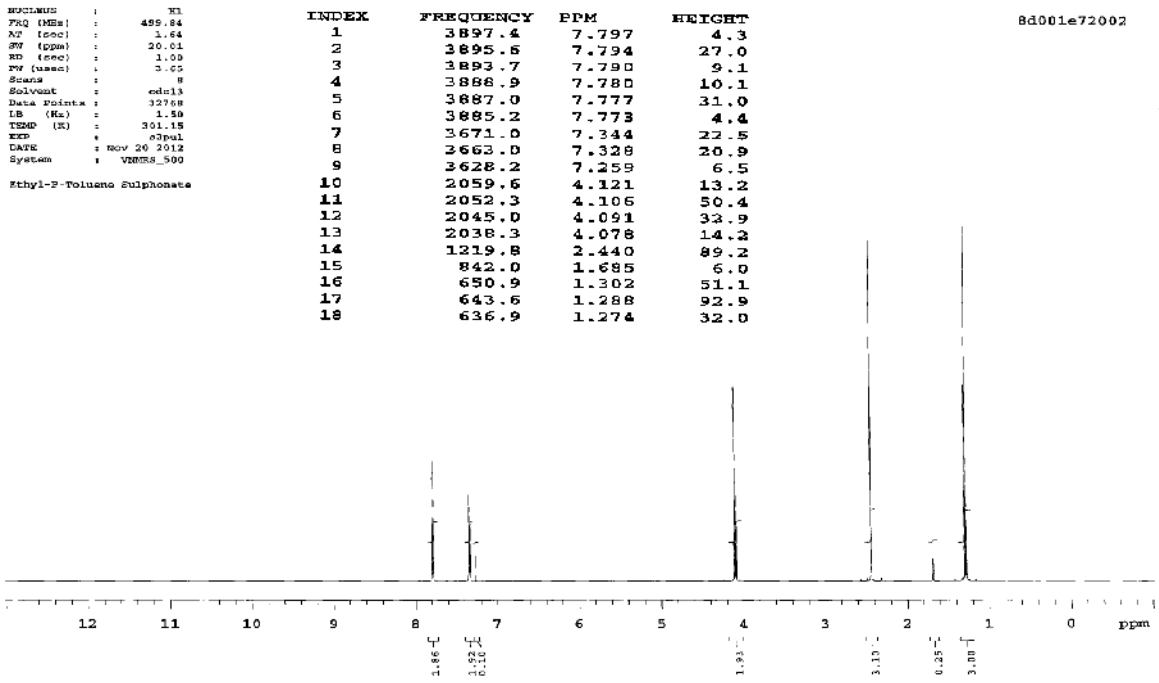

Fig. 11. Proton NMR for Ethyl Tosylate.

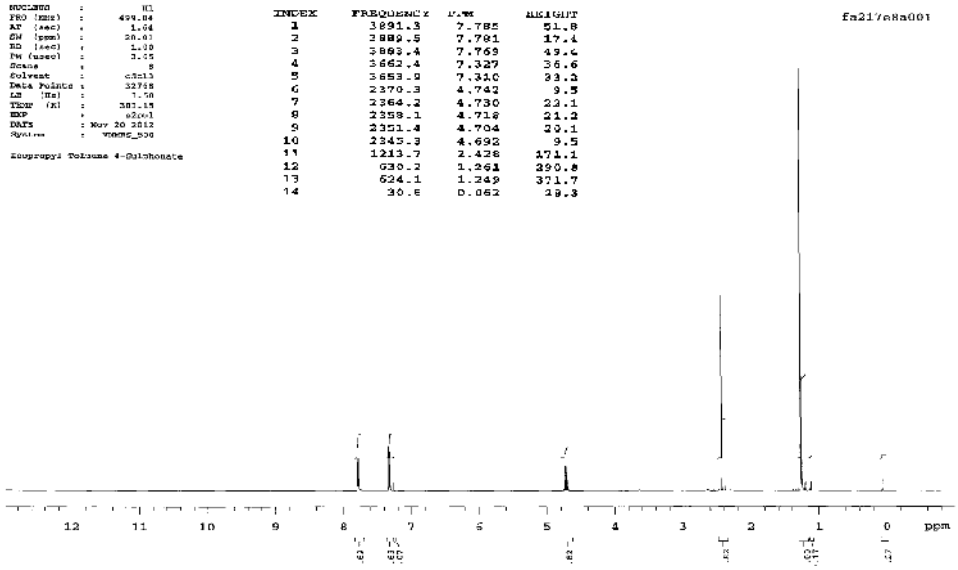

Fig. 12. Proton NMR for Isopropyl Tosylate.

The separation was achieved using Sodium perchlorate buffer and acetonitrile in the ratio of (50:50). The concentration of buffer was optimized for better peak shape of Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate.

Sodium perchlorate buffer with concentration of 50mM with different composition of acetonitrile was employed. In the next approach mixture of Sodium perchlorate buffer and acetonitrile in the ratio of (60:40 v/v/) using RRHD Eclipsed Plus C18 (50 x 2.1mm, 1.8µm) column. Under these condition Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate impurities eluted in close proximity to Sorafenib's other unknown impurities. With decrease of Acetonitrile content and increased content of buffer, the required resolution was obtained.

B. Method validation

1. Specificity. Specificity of the method is its ability to detect and separate all the impurities present in the drug. Specificity of the method is demonstrated in terms of spectral as well as peak purity data of the drug and impurities present in drug. Identification solution of Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate were injected at working level concentration along with Sorafenib Tosylate 15mg/ml. A spiked sample of Sorafenib Tosylate with Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate was also injected. The peaks of Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate were well resolved from each other (Fig.13) as well as Sorafenib and its other impurities. Spectral data confirmed that the peaks were pure and there was no interference at the Rt of Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate.

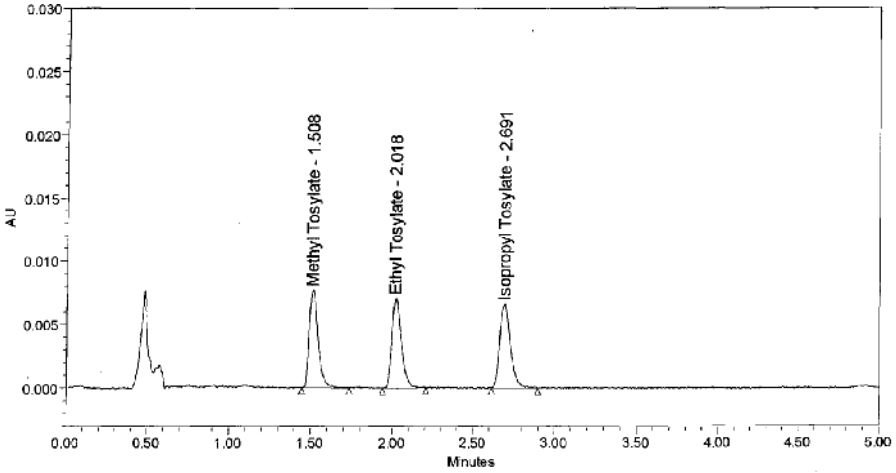

Fig. 13. Standard chromatogram.

2. Linearity. Linearity of the method was checked by preparing solutions at six concentration levels of 0.09 ppm (Level 1), 0.1 ppm (Level 2), 0.15 ppm (Level 3), 0.2 ppm (Level 4), 0.25 ppm (Level 5) and 0.3 ppm (Level 6) for Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate. Level 1 and level 6 was injected six times were as level 2, level 3, level 4 and level 5 was injected two times. The mean responses recorded for each impurity were plotted against concentration. The correlation coefficient for Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate was found to be 0.9996, 0.9999 and 1.0000 respectively, which indicates good linearity. The %Y intercept for Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate was found to be 2.64, -0.97 and 0.37 respectively (Fig. 14).

3. Accuracy

Sorafenib samle solution solution of 15mg/ml was spiked with each impurity solution of Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate at different concentration of 0.09, 0.1, 0.2 and 0.3 ppm. Each Level solution was prepared in triplicate and injected. The recovery percentage and %R.S.D were calculated for each impurity. Recovery of Methyl Tosylate ranged from 96.6-101.1%, 99.0-105.0%, 106.6-109.3% and 100.7-105.7%, Ethyl Tosylate ranged from 112.2-114.6%, 99.0-100.0%, 102.0-106.6% and 100.3-108.4% and Isopropyl Tosylate ranged from 101.9-109.9%, 104.0-109.1%, 100.5-105.5% and 97.9-104.3% respectively. The results are shown in Table 1 respectively.

The acceptance criteria for recovery of an impurity at a concentration level of 0.09 ppm is between 85% and 115% and at 0.1, 0.2 and 0.3 ppm is 90% to 110% (Fig. 15).

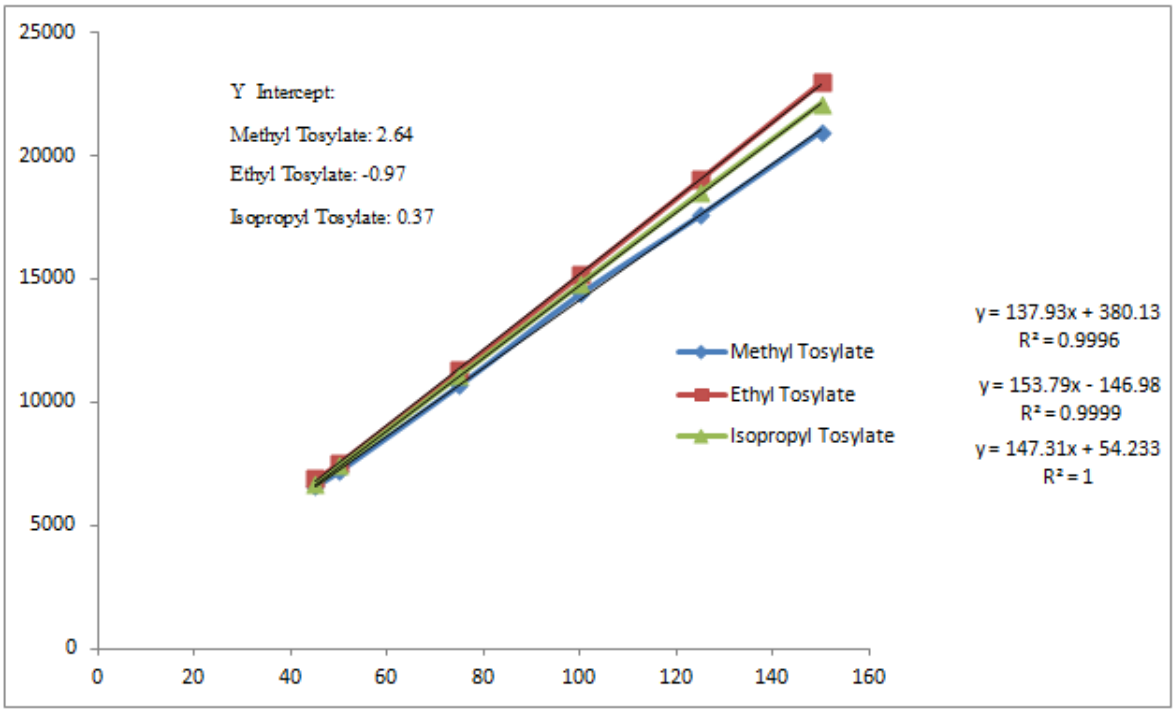

Fig. 14. Linearity data for Methyl Tosylate, Ethyl Tosylate and Isopropyl Tosylate.

Table 1. Accuracy results for Methyl Tosylate, Ethyl Tosylate and Isopropyl Tosylate.

Added (µg)	Recovered (µg)	%Recovery	%RSD
0.09	0.087	96.6	2.24
	0.089	98.8	
	0.910	101.1	
0.10	0.105	105.0	2.94
	0.102	102.0	
	0.099	99.0	
0.20	0.211	106.6	1.70
	0.213	107.6	
	0.218	109.3	
0.30	0.314	105.7	3.64
	0.321	108.1	
	0.299	100.7	

Accuracy results of Ethyl Tosylate.

Added (µg)	Recovered (µg)	%Recovery	%RSD
0.09	0.10	114.6	1.01
	0.098	112.2	
	0.099	113.4	
0.10	0.098	99.9	0.55
	0.098	99.0	
	0.099	100.0	
0.20	0.211	106.6	2.21
	0.202	102.0	
	0.208	105.0	
0.30	0.322	108.4	3.87
	0.310	104.3	
	0.298	100.3	

Accuracy results of Isopropyl Tosylate.

Added (µg)	Recovered (µg)	%Recovery	%RSD
0.09	0.096	109.9	3.91
	0.091	104.2	
	0.089	101.9	
0.10	0.108	109.1	2.50
	0.107	108.1	
	0.103	104.0	
0.20	0.209	105.5	3.11
	0.211	106.6	
	0.199	100.5	
0.30	0.310	104.3	3.17
	0.299	100.7	
	0.291	97.9	

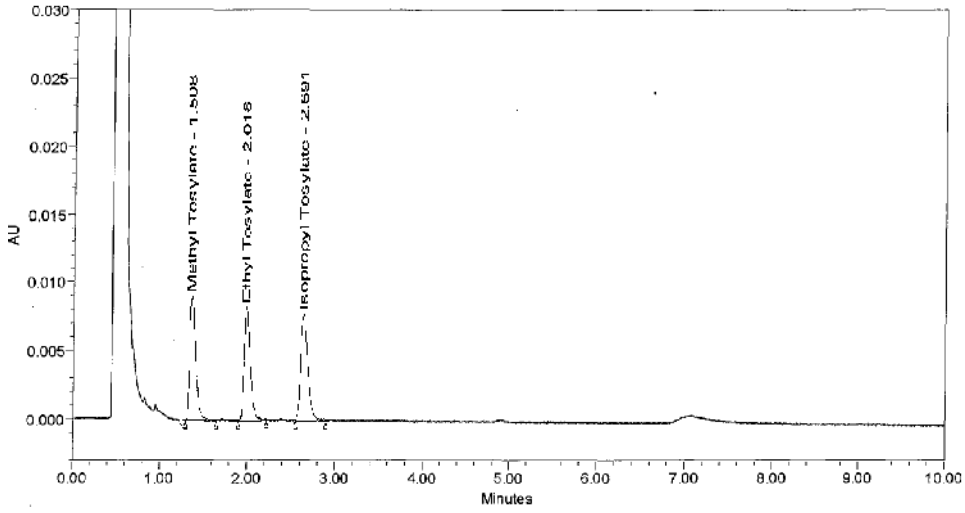

Fig. 15. Accuracy Chromatogram.

4. Limit of detection

The sensitivity for detection can be demonstrated by determining the limit of detection (LOD). A signal to noise (S/N) ratio between 3 to 10 is generally considered to be acceptable for estimating detection limit.

S/N ratios of individual peak were determined at different concentration at estimate LOD and respective % RSD was calculated for replicate injection (n = 3). The LOD was found to be 0.045 ppm for Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate. The results are shown in the Table 2.

5. Limit of quantification

The quantification limit is the lowest concentration of a substance that can be quantified with acceptable precision and accuracy. A typical S/N ratio of 10-30 is generally considered to be acceptable for estimating the limit of quantification. S/N rations of individual peaks were determined at different concentration to estimate limit of quantitation (LOQ) and respective %R.S.D was calculated for replicate injection (n = 6). The LOQ was determined to be 0.09 ppm for Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate. The results are shown in Table 2.

Table 2. Lod and Loq Results of Impurities.

Compound Name	LOD(ng/ml)	S/N Ratio	LOQ(ng/ml)	S/N Ratio	%RSD
Methyl Tosylate	0.04	4	0.09	12	3.67
Ethyl Tosylate	0.04	5	0.09	11	3.89
Isopropyl Tosylate	0.04	4	0.09	12	2.98

6. System and method precision

The precision for Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate in Sorafenib was checked by forming repeatability. The sample was prepared by spiking Sorafenib with the solution of Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate a concentration of 0. 2 ppm and injected six times. The % R.S.D was found to be less than 1.3% for content of Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate for six replicate injections.

To determine the method precision six independent solution were prepared by spiking Sorafenib with the solution of Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate a concentration of 0. 2 ppm. Each solution was injected once. The variation in the results for the content of Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate were expressed in terms of % R.S.D. The values calculated were 2.8%, 1.6% and 2.2% respectively for Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate, indicating satisfactory method precision.

IV. SAMPLE PREPARATION OF SORAFENIB TOSYLATE FOR ROUTINE ANALYSIS

Weighed 150mg of Sorafenb Tosylate sample in 10ml volumetric flask, dissolved in 4ml of acetonitrile and dilute up the volume with buffer. Filtered this solution using 0.45µ syringe filter. Injected this solution into UPLC to determine the amount of impurities present in the sample. Three different batches of Sorafenib Tosylate was analyzed under developed condition and presented the results in Table 3**.** The chromatogram obtained after the analysis was shown in (Fig. 16)**.**

Table 3. Results Obtained Form Three Batches of Sorafenib Tosylate.

Compound name	Methyl Tosylate	Ethyl Tosylate	Isopropyl Tosylate
B.No. A	Not Detected	Not Detected	Not Detected
B.No. B	Not Detected	Not Detected	Not Detected
B.No. C	Not Detected	Not Detected	Not Detected

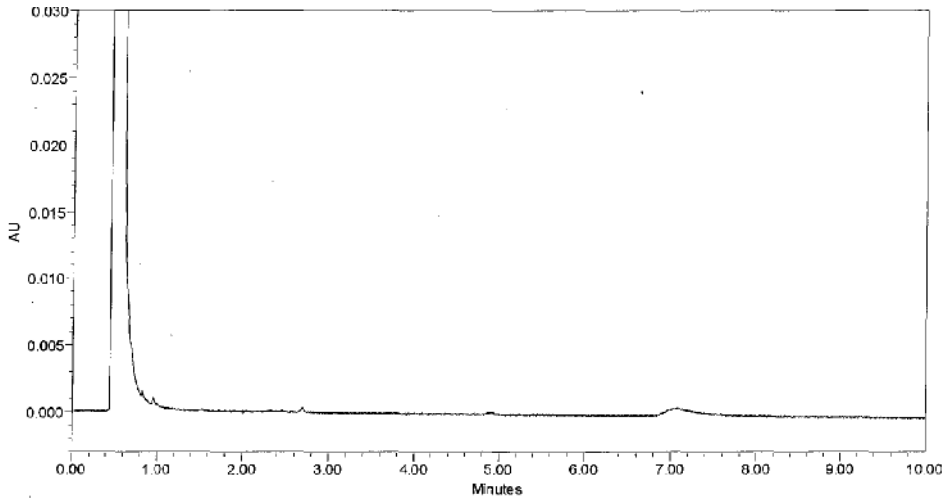

Fig.16. Sample Chromatogram.

V. CONCLUSION

The proposed LC method is selective for the quantification of Genotoxic Methyl Tosylate, ethyl Tosylate and Isopropyl Tosylate present in Sorafenib. The method is capable of detecting three process impurities. Hence this method is useful for the detection of Potential Genotoxic impurities present in Sorafenib Tosylate.

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